Possible Compartmentalization of Hepatitis C Viral Replication in the Genital Tract of HIV-1–Coinfected Women

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Background. We estimated the prevalence of hepatitis C virus (HCV) in cervical cytobrush samples from HCV/human immunodeficiency virus (HIV)–coinfected women and analyzed the HCV quasi species in both cytobrush and plasma samples. Possible compartmentalization of viral quasi species in the genital tract and plasma was evaluated by comparison of genetic heterogeneity and use of phylogenetic analysis.

Methods. Paired plasma and cytobrush samples were obtained from 85 HCV/HIV-coinfected women. The presence of HCV in cytobrush samples was evaluated by reverse-transcription polymerase chain reaction of the 5′ untranslated region. Viral quasi species were analyzed by cloning and sequencing the highly variable region–1 in 8 patients.

Results. HCV was detected in 27% of cytobrush samples. The composition of viral quasi species was different in the 2 body compartments at both the nucleotide and amino acid level. In fact, the mean complexity was significantly lower in cytobrush samples, and a similar trend was observed for the other parameters of heterogeneity. Phylogenetic analysis and amino acid alignment identified several viral variants that were unique to each body compartment.

Conclusions. Our data suggest that the genital and plasma quasi species represent distinct subpopulations, which possibly reflects compartmentalized viral replication. Alternatively, cell carriers harboring viral quasi species in the genital tract that are distinct from those in plasma could transfer the virus through the barrier separating the 2 body sites.

Hepatitis C virus (HCV) infection, in addition to causing liver disease, is associated with a number of extrahepatic manifestations, some of which have been related to the ability of HCV to infect and localize in cells and tissues other than the liver, particularly hematopoietic cells [1–5]. As a matter of fact, HCV has been detected in saliva, spinal and seminal fluid, and vaginal secretions [6–11], although the source of virus at these sites is unknown. Extrahepatic sites where compartmentalized HCV replication occurs may be a viral reservoir from which viral replication can eventually resume after therapeutic success or after orthotopic liver transplantation, and they may be involved in the transmission of minor quasi species components in sexual or vertical infection [7, 8, 12–15].

HCV and HIV share common pathways of transmission, and rates of sexual transmission of HCV may be higher from coinfected partners [16]. In this respect, the prevalence and evolution of HCV in the genital tract of women who also have HIV infection has only recently been considered. Recently, HCV has been detected in genital secretions from HIV-positive women in a proportion very similar to that in previous analyses that included HIV-negative women (~30%) [17–19]. From these results, the HCV genome in female genital secretions was presumed to be associated with the cellular fraction, which is consistent with cell-mediated carriage from the circulation. However, because negative-strand HCV RNA has been detected in cervicovaginal lavage samples [17], it is likely that the virus found in this extrahepatic site may also result from replication events occurring in resident cells.
In the present study, we evaluated the prevalence of HCV in cervical cytobrush samples from women coinfected with HIV, and we compared the HCV quasi species present in cytobrush and plasma samples. To highlight the genetic relationships between viral quasi species present in these body compartments and to detect the possible compartmentalization of viral variants, quantitative parameters of genetic heterogeneity were compared, and phylogenetic trees and amino acid sequences of the highly variable region (HVR)–1 were analyzed.

**PATIENTS, MATERIALS, AND METHODS**

**Patients.** Eighty-five patients attending the outpatient clinic at the National Institute for Infectious Diseases “L. Spallanzani,” Rome, for human papillomavirus (HPV) monitoring were retrospectively selected, on the basis of a known positivity to HCV antibody, from a larger cohort of 350 HIV-positive women. The cervical cytobrush–Ayres spatula sample was used to prepare the Pap smears, immersed in a tube that contained 1 mL of PBS, and gently agitated. The liquid was then aliquoted in 2 tubes and stored at −80°C until use. One aliquot was used for the detection and typing of HPV, and the remaining aliquot was used for the present study. All patients provided signed, informed consent for the use of their samples for research purposes. The collection of personal data and behavioral information was obtained using a questionnaire. The study was authorized by the institutional ethics committee. HCV viremia data were available for 80 of the HCV antibody–positive women: 63 had measurable HCV RNA (median, 5.70 log_{10} IU/mL; range, 2.87–6.83 IU/mL); 17 women had HCV RNA loads below the lower limit of detection of the quantification assay (i.e., <2.78 log_{10} IU/mL). For the remaining 5 patients, viremia data were not available.

Screening for the presence of HCV RNA in cervical cytobrush samples was performed for all 85 women from this subgroup, and 23 tested positive. The analysis of HCV quasi species in plasma and cytobrush samples was completed for 8 patients. Of these, 2 were infected with HCV genotype 1a, and 6 were infected with HCV genotype 3a.

**Virological assessment.** Screening for plasma HCV RNA was performed using qualitative HCV RNA detection by polymerase chain reaction (PCR; limit of detection, 50 IU/mL) (Amplipcr; Roche Diagnostic Systems). The viral load in positive plasma samples was determined by quantitative reverse-transcription (RT)–PCR (HCV Amplipcr Monitor) with a linear range of 6 × 10²–5 × 10⁴ IU/mL. When the specimen volume was sufficient, plasma samples with values exceeding the linear range of the assay were retested after a 1:100 dilution. HCV genotyping was done using a reverse-hybridization line probe assay (INNO-LiPA HCV; Innogenetics) based on the 5′ noncoding region (NCR).

**RNA extraction and RT-PCR conditions.** RNA was extracted from plasma and cervical cytobrush samples using the method of Boom et al. [20]. The presence of inhibitors from the sample matrix was ruled out by testing the presence of β-globin DNA in all extracts from cytobrush samples.

RT of total RNA was performed using a reverse transcriptase kit (Invitrogen) in accordance with the manufacturer’s instructions. Amplification of the 5′ NCR from cervical cytobrush samples was done using nested PCR protocols, including TaqGold DNA polymerase (Applied Biosystems). Ten microliters of cDNA was amplified in the first round of amplification using the primers 5′-AACTACTGTCTTCACGCAGAA-3′ (sense; positions 16–36, HCV clone MD2b10-2, GenBank accession number AY232749.1) and 5′-GATGACCGTCTACAGAGACCTC-3′ (antisense; positions 304–283, HCV clone MD2b10-2, GenBank accession number AY232749.1). Amplification conditions were as follows: denaturation for 15 min at 94°C, then 34 cycles of 30 s at 94°C, 30 s at 45°C, and 1 min at 72°C. The amplicon size for the primary reaction was 290 bp. An aliquot (2.5 μL) of the first-round reaction was used for the second round of amplification, using the internal primers 5′-ATGGCGTTAGTGAGTGT-3′ (sense; positions 48–65, HCV clone MD2b10-2, GenBank accession number AY232749.1) and 5′-GGGACCCAAAACATACGCGCT-3′ (antisense; positions 202–223, HCV clone MD2b10-2, GenBank accession number AY232749.1). Amplification conditions were as follows: denaturation for 15 min at 94°C, then 24 cycles of 30 s at 94°C, 30 s at 45°C, and 30 s at 72°C. The nested amplicon size was 175 bp. Amplification of HVR-1 in RNA from plasma and vaginal fluids was performed with nested PCR protocols, using primers that targeted the aminoterminal region of the E2 gene for the different genotypes. PCR conditions for HVR-1 genotypes 1b and those for genotypes 1a, 2, and 3 were different and were derived from the literature [21, 22].

Positive and negative controls, which contained standardized viral RNA extracts and nuclease-free water, respectively, were included in each RT-PCR assay. The amplified products were analyzed by agarose gel electrophoresis and visualized by UV fluorescence after staining with ethidium bromide.

**HVR-1 cloning, sequencing, and quasi species assessment.** The HVR-1 region was cloned using nested PCR products. Amplified products were purified using the QIAquick PCR Purification kit (Qiagen), ligated into the pCR4-TOPO vector contained in the TOPO TA Cloning kit (Invitrogen Life Technologies), and transformed in competent *Escherichia coli* cells using the One Shot TOP10 system (Invitrogen Life Technologies). Plasmid DNA was extracted using the QIAprep Miniprep kit (Qiagen). A total of 160 HVR-1 clones (5–16 clones from each sample) were sequenced on ABI Prism 3100 using the BigDye Terminator cycle sequencing kit (Applied Biosystems).

Quasi species analysis was performed on an 81-bp nucleotide sequence in the E2 region of the HVR-1 gene (positions
Determination of the presence of HCV in cytobrush samples from HIV-coinfected women. To assess the presence of HCV in cytobrush samples from HIV/ HCV-coinfected women, nested PCR specific for the 5' NCR was performed for samples from 85 patients attending the outpatient clinic at the National Institute for Infectious Diseases “L. Spallanzani,” Rome. Of these, 23 (27%) had HCV sequences present in cytobrush samples. In particular, 22 (96%) of 23 cytobrush-positive women had measurable circulating HCV RNA (median, 5.70 log$_{10}$ IU/mL; range, 4.38–6.20 log$_{10}$ IU/mL), and only 1 had a circulating HCV RNA load <$2.78$ log$_{10}$ IU/mL. Of the 62 cytobrush-negative women, 41 (66%) had measurable HCV RNA in plasma (median, 5.70 log$_{10}$ IU/mL; range, 2.87–6.83 log$_{10}$ IU/mL), and 16 had HCV RNA loads <$2.78$ log$_{10}$ IU/mL (table 1). For the remaining 5 cytobrush-negative women, HCV viremia information was lacking. Table 1 shows that the proportion of HCV-positive cytobrush samples was significantly higher (34.9%) in women with measurable HCV viremia than in those with HCV RNA loads <$2.78$ log$_{10}$ IU/mL (5.9%) ($P = .041$). However, median HCV RNA loads did not differ between women with or without HCV RNA present in the cytobrush sample (5.7 log$_{10}$ IU/mL in both groups).

**HVR-1 heterogeneity analysis of plasma and genital-tract HCV.** To establish the heterogeneity of HCV in the 2 body compartments, a subset of 8 women with HCV-positive cytobrush samples and plasma viral loads $>$2.78 log$_{10}$ IU/mL were selected, and their clinical features are described in table 2. Genetic heterogeneity of the HVR-1 of the E2 gene was investigated using a cloning and sequencing approach. A total of 98 plasma and 79 cytobrush clones were analyzed. The complexity, diversity, and proportion of $K_s$ and $K_a$ for each subject and for each compartment, as well as median values, are shown in table 3. The complexity, which reflects the number of different variants present in the analyzed compartment, was significantly lower in cytobrush samples than in plasma samples at both the nucleotide and amino acid level. Diversity, $K_s$, $K_a$, and the $K_a/K_s$ ratio also tended to be lower in cytobrush samples than in plasma samples. This tendency was particularly evident in patients B, C, and D and was reflected in mean values, although the differences were not statistically significant (table 3).

**HVR-1 quasi species compartmentalization in plasma and cytobrush samples.** With the aim of evaluating compartmentalized viral replication of HCV from cytobrush samples, compared with that from plasma samples, phylogenetic trees were constructed on the basis of nucleotide sequences. Figure 1 shows the phylogenetic tree of all clones from the 8 patients. As expected, the clone sequences from the different patients segregated independently from each other, indicating the absence of cross-contamination between the samples and then remaining associated with their respective genotype (1a and 3a). It should be noted that clones from patients G and F formed 2 very homogeneous, independent clusters. Information about the duration of HCV infection was available only for patient G (≥4 years). The high homogeneity of clones from patients G and F was consistent with the corresponding values of heterogeneity parameters (table 3). This table shows that, in each patient, cytobrush and plasma samples varied in the composition of viral quasi species, with some clones represented in both and others represented in only 1 of the 2 body sites. In

<table>
<thead>
<tr>
<th>HCV load</th>
<th>Positive, no. (%)</th>
<th>Negative, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&gt;$2.78 log$_{10}$ IU/mL</td>
<td>22 (34.9)</td>
<td>41 (66.1)</td>
</tr>
<tr>
<td>$&lt;$2.78 log$_{10}$ IU/mL</td>
<td>1 (5.9)</td>
<td>16 (94.1)</td>
</tr>
</tbody>
</table>

* $P = .041$, $\chi^2$ test.
which indicates the possibility that women who are virtually HCV RNA load below the limit of quantification of our assay, also detected in a cytobrush sample from a woman with an higher in women with measurable HCV RNA loads, but it was women [18].

en [17] and to that from a previous report of HIV-negative similar to that recently reported in HCV/HIV-coinfected wom-

harbor HCV in their genital tract. This prevalence was very plasma, to explore the possibility that female genital tract can present in this body compartment, compared with those in coinfected women, and we analyzed the HVR-1 quasi species sequences in cervical cytobrush samples from HCV/HIV-

DISCUSSION
In the present study, we evaluated the prevalence of HCV ge-

nome sequences in cervical cytobrush samples from HCV/HIV-

coinfected women, and we analyzed the HVR-1 quasi species present in this body compartment, compared with those in plasma, to explore the possibility that female genital tract can harbor viral quasi species different from those in plasma. Approximately one-quarter of these women were found to harbor HCV in their genital tract. This prevalence was very similar to that recently reported in HCV/HIV-coinfected women [17] and to that from a previous report of HIV-negative women [18].

The frequency of HCV in cytobrush samples was significantly higher in women with measurable HCV RNA loads, but it was also detected in a cytobrush sample from a woman with an HCV RNA load below the limit of quantification of our assay, which indicates the possibility that women who are virtually without viremia may harbor HCV in other body compartments. These results are not unanticipated, given that previous reports have shown that, in patients negative for HCV RNA, HCV may be present in other body sites, such as the gastric mucosa [23].

We were unable to measure the HCV RNA loads in cytobrush samples using commercial methods, because of the scarcity of available material. However, the HCV genome was detected by an in-house method targeting the 5' NCR, and the HVR-1 region could be successfully amplified as well. The availability of HVR-1 amplicons prompted the use of a cloning and sequencing approach based on a coding region, so that the corresponding amino acid sequences could be deduced. This region is of particular interest when studying HCV quasi species, because it is under the selective pressure of host immune re-

response and is the main focus of interest when analyzing viral dynamics and evolution. In addition, we could determine not only the complexity and diversity but also \( K_a \) and \( K_s \), whose ratio is informative with respect to the extent of immunologic pressure. In fact, it is generally assumed that a \( K_a/K_s \) ratio \( >1 \) indicates the existence of immunologic pressure on the evolution of viral quasi species.

Our results indicate that the heterogeneity of viral quasi species was indeed different in plasma and cytobrush samples at both the nucleotide and amino acid levels. The mean complexity in cytobrush samples was significantly lower (table 3), and a similar trend was also observed for diversity, \( K_a \), \( K_s \), and \( K_a/K_s \) values, although the differences did not reach statistical significance. When considering the phylogenetic trees, viral variants were identified in the background of several common clones at both the nucleotide and amino acid level that were unique of either body compartment.

Our findings extend the results of Nowicki et al. [17], who recently showed, on the basis of the sequence analysis of the 5' NCR region, compartmentalization of HCV in the blood and genital tract of HCV/HIV-coinfected women, which suggests the possible localized replication of HCV in the genital tract. Our data show, to our knowledge for the first time, a different composition of viral quasi species based on the coding region that includes epitopes under immunologic pressure. For both

<table>
<thead>
<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug use</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>UNK</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Age, years</td>
<td>46</td>
<td>40</td>
<td>37</td>
<td>39</td>
<td>53</td>
<td>38</td>
<td>35</td>
<td>UNK</td>
</tr>
<tr>
<td>Plasma HCV load, log_{10} IU/mL</td>
<td>&gt;5.70</td>
<td>6.05</td>
<td>6.16</td>
<td>5.85</td>
<td>5.68</td>
<td>4.94</td>
<td>4.72</td>
<td>&gt;5.70</td>
</tr>
<tr>
<td>HCV genotype</td>
<td>3a</td>
<td>3a</td>
<td>1a</td>
<td>1a</td>
<td>3a</td>
<td>3a</td>
<td>3a</td>
<td>3a</td>
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<tr>
<td>HIV stage</td>
<td>NA</td>
<td>C3</td>
<td>B3</td>
<td>NA</td>
<td>B2</td>
<td>NA</td>
<td>B3</td>
<td>C3</td>
</tr>
<tr>
<td>ALT level, mU/mL</td>
<td>NA</td>
<td>36</td>
<td>35</td>
<td>NA</td>
<td>126</td>
<td>NA</td>
<td>23</td>
<td>149</td>
</tr>
<tr>
<td>Inflammation detected on the Pap test</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

NOTE. ALT, alanine aminotransferase; HCV, hepatitis C virus; NA, not available; UNK, unknown.
Table 3. Heterogeneity of the highly variable region–1 quasi species in plasma (P) and cytobrush (C) samples.

<table>
<thead>
<tr>
<th>Patient, sample (no. of clones)</th>
<th>Complexity (S&lt;sub&gt;n&lt;/sub&gt;)</th>
<th>Mutational changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At the amino acid level</td>
<td>At the nucleotide level</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (10)</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>P (10)</td>
<td>0.70</td>
<td>0.98</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (16)</td>
<td>0.48</td>
<td>0.50</td>
</tr>
<tr>
<td>P (13)</td>
<td>0.94</td>
<td>0.99</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (9)</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>P (12)</td>
<td>0.76</td>
<td>0.82</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (12)</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>P (12)</td>
<td>0.69</td>
<td>0.82</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (5)</td>
<td>0.31</td>
<td>0.59</td>
</tr>
<tr>
<td>P (13)</td>
<td>0.34</td>
<td>0.50</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (6)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>P (16)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (11)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>P (15)</td>
<td>0.14</td>
<td>0.23</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (10)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>P (7)</td>
<td>0.49</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Total, mean ± SE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.18 ± 0.06</td>
<td>0.22 ± 0.08</td>
</tr>
<tr>
<td>P</td>
<td>0.51 ± 0.12</td>
<td>0.62 ± 0.013</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td><strong>0.026</strong></td>
<td><strong>0.020</strong></td>
</tr>
</tbody>
</table>

**NOTE.** NA, not applicable.

<sup>a</sup> Mean no. of nucleotide substitutions per site.

<sup>b</sup> Mean proportions of synonymous substitutions per synonymous site.

<sup>c</sup> Mean proportions of nonsynonymous substitutions per nonsynonymous site.

<sup>d</sup> Mean K<sub>s</sub>: mean K<sub>s</sub> ratio.

<sup>e</sup> Comparisons between means, using Student’s t test.

Fluids, the K<sub>s</sub>: K<sub>s</sub> ratio was >1, which confirms that the evolution of this region is driven by immunologic pressure in both compartments. In addition, because K<sub>s</sub>: K<sub>s</sub> values in plasma were higher than those in the genital tract, the extent of such pressure seemed to be higher in the circulation, which is possibly due to a higher exposure of HCV to the effector arms of the immune response. In fact, if HCV actually replicates in the genital mucosa, it is reasonable that immune pressure in this area is lower than that in circulation. However, further experiments are necessary to substantiate this hypothesis—possibly those based on in situ hybridization or RT-PCR of biopsy samples from the endocervical lining, which would be more suitable for obtaining information about local reservoirs of HCV in the genital tract. In this respect, the quasi species analysis of HCV present in body sites other than blood in the uncommon persons without detectable HCV RNA loads will be highly informative about the extent of immunologic pressure in local compartments and will be the focus of further studies.

In addition, our data show that the qualitative composition of viral quasi species in individual patients is different in plasma and cytobrush samples, given that unique sequences were identified in the 2 body sites, with monophyletic segregating clusters observed only in plasma. In a recent study of the HVR-1 region in seminal fluid from HCV/HIV-coinfected patients, Briat et al. [24] described the presence of unique variants in blood and semen, which suggests that HCV that is shed into the male genital tract originates from a preferential transfer of viral particles from blood into genital secretions.

On the basis of the our data, it is not possible to establish whether the different composition of viral quasi species in the
Figure 1. Phylogenetic tree of nucleotide sequences for all patients. Nucleotide sequences of the highly variable region (HVR)–1 as it relates to the 1a and 3a genotypes (hvr1-1a and hvr1-3a) were also included. Nos. at nodes indicate the frequency (%) of their occurrence in bootstrap analysis based on 500 replicates. Values ≥80% are shown and were considered to be significant. Patients are identified by different colors. Circles, plasma clones; triangles, cytobrush clones.
Figure 2. Phylogenetic trees (upper panels) and alignment of amino acid sequences (lower panels) of the highly variable region 1 of patients A, B, C, D, and E. Nos. at nodes indicate the frequency (%) of their occurrence in bootstrap analysis based on 500 replicates. Values >80% are shown and were considered to be significant. For amino acid alignments, 1 arbitrarily selected sequence is entirely shown, and position identities are shown as dots. Plasma and cytobrush sequences are indicated as P and C, respectively; nos. in brackets indicate the nos. of the corresponding clones (cl). Sequences unique to plasma samples are in boxes, and sequences unique to cytobrush samples are shaded gray. In the phylogenetic tree: circles, plasma clones; triangles, cytobrush clones.
genital tract of HCV/HIV-coinfected women could actually reflect compartmentalized viral replication or the existence of selective mechanism(s) controlling the transfer of plasma quasi species through the barrier separating the 2 compartments. It should be noted that the cytoplasmic samples for the quasi species analysis were evidently not contaminated by blood, so it is reasonable to assume that HCV in cytoplasmic samples did not derive merely from blood contamination of the specimen. Inflammation was present in 4 women (table 2), so it is possible that circulating mononuclear cells harboring HCV infection can cross the anatomical barrier between plasma and the female genital tract and, thus, act as HCV carriers. However, in 2 women with inflammation (patients B and C), cytoplasmic-specific variants were observed (figure 2). However, there is also the possibility that localized replication of HCV occurs in cell reservoirs resident in the genital mucosa, leading to an independent evolution of HCV quasi species. In fact, for HCV present in cerebrospinal fluid (CSF), a clear segregation of 5′ untranslated region variants has been observed, which is suggestive of local replication [25]. Moreover, on the basis of similarity between HCV variants from CSF and peripheral blood mononuclear cells (PBMCs), it has been postulated that PBMCs may carry the virus into the brain and that, thereafter, resident microglial cells maintain viral replication.

In conclusion, independent of the mechanisms involved in the presence of HCV in the female genital mucosa, our data confirm and extend previous evidence that viral quasi species in this specific body compartment contain components different from those that circulate. This finding may have pathogenic implications with respect to the possible perinatal and sexual transmission of distinct viral variants.

Acknowledgment

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References


